With the compliments of S. Akai

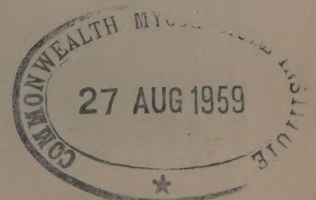
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On the resistance of cucumber varieties to anthracnose and the behavior of the causal fungus in the invasion of the host tissues[a]

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Introduction

The anthracnose fungus, *Colletotrichum lagenarium*, attacks almost all varieties and species of Cucurbitaceae often causing severe damage, especially to watermelon and cucumbers. According to Gardner,[1] cucumbers are generally susceptible whereas squashes (*Cucurbita moschata* and *C. maxima*) are resistant. Recently, Kawai[3] called attention to varietal resistance of watermelon to anthracnose. Iwata[2] found that triploid cucumbers were more resistant than the diploid ones, and concluded that the tetraploid cucumbers, produced by the colchicine treatment, were more resistant than the ordinary diploid ones. He made no attempt to analyze the nature of resistance.

The present investigation was made during 1953 and 1954 in order to determine the nature of resistance in cucumbers to the anthracnose fungus.

Materials and methods

C. lagenarium was isolated in August, 1951, by Yasumori from naturally infected leaves of cucumber. The conidia for inoculation and germination tests were obtained from the 8-11 day-old culture grown on a corn meal agar slant at 24° C. Redistilled water was always used in making conidia suspensions.

Twenty-eight varieties[b] of cucumber and twenty varieties[b] of squash were used. Each was planted in a 12 cm. pot and raised in the greenhouse. When the plants grew to about the five-leaf stage, side branches and tendrils were cut off and the conidial suspension was sprayed on the upper surface of leaves with an atomizer. The pots were transferred into a moist chamber at 24° C. for 24 hours, then they were taken out and placed in the greenhouse. After 6 days, when definite spots developed, the total number of spots and area of leaves were measured.

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a) Contributions from the Laboratory of Phytopathology, Kyoto University, No. 94.
We are indebted to Dr. Imamura, Professor of Applied Botany, Kyoto University, and Dr. J. J. Christensen, Professor of Plant Pathology, Minnesota University for his kind suggestions and help.
b) Seeds were sent from the Kyushu, Osaka, Shimane, Shizuoka and Hyogo Agricultural Experiment Stations, and the Aichi Horticultural Experiment Station. We wish to express our best thanks.

Experimental results

All varieties of squashes tested were highly resistant to *C. lagenarium*, whereas some varieties of cucumbers were resistant and others susceptible. The varietal reaction of cucumbers to anthracnose is shown in Table 1. Although these data presented a wide

Table 1. Reaction of cucumber varieties to *Colletotrichum lagenarium* as indicated by the number of lesions produced on the leaves.

Varieties	Number of spots per unit area (cm^2) of leaf [1]		
	1st inoculation	2nd inoculation	Average
Tsuda-Sanjaku	0.19	0.39	0.29
Sekino No. 2	0.19	0.40	0.30
Taiwan-Kema	0.30	0.37	0.34
Wild cucumber	0.36	0.35	0.36
Sakata-Pickle	0.27	0.60	0.44
Ochiai	0.20	0.89	0.55
Shina-Sanjaku	0.32	0.79	0.56
Chiba-Ao-Hushinari No. 2	0.36	0.81	0.59
Ko-Jo	0.22	1.03	0.63
Kaga-Ao-Hushinari	0.58	0.75	0.67
Sekino No. 1	0.60	0.90	0.75
Seki-Jo	0.39	1.14	0.77
Kurume-Ochiai	0.52	1.02	0.77
Ochiai-Ao-Hushinari	0.38	1.23	0.81
Yamato	0.45	1.22	0.84
Sekino No. 3	0.70	1.07	0.89
Pekin	1.04	1.67	1.36
Sagami-Hanpaku-Hushinari	1.15	1.87	1.51
Shiro-Hushinari No. 26-4	1.26	2.18	1.72
Dai-Hasshi	1.07	2.45	1.76
Shiro-Hushinari No. 32-5	1.59	2.37	1.98
San-To	1.95	2.36	2.29
Su-Yo	2.11	2.71	2.41
Nisshi-Aonaga	1.66	3.74	2.70
Kwachu-Zairai	2.89	4.40	3.65

1) Three to four plants per variety, each plant having three to six, average five leaves were tested.
The first inoculation was made on Sept. 1, 1953, and the second on Sept. 2.

difference in the number of spots per unit area of leaf among the varieties, it is virtually impossible to divide the varieties into two distinct groups, resistant and susceptible. Even resistant varieties developed a considerable number of lesions on their leaves. The data on the rate of enlargement per day of individual lesions are presented in Table 2. The spots were measured four or five times during a period from 7 to 14 days after inoculation. The enlargement of lesions occurred at about the same rate regardless of the varieties involved.

These results indicate that the difference in susceptibility (Table 2) of cucumber varieties is related to the number of spots produced per leaf, rather than to rapidity of

Table 2. The rate of enlargement of individual anthracnose lesions produced on mature leaves of cucumber

Variety	Average enlargement rate of diameter of individual leasion mm per day
Wild cucumber [1]	1.1
Sekino No. 2 [1]	1.8
Su-Yo [2]	1.2
Kwachu-Zairai [2]	2.0

1) A small number of lesions was produced when inoculated.
2) Abundant lesions were formed, see Table 1.

lesion enlargement. Hence an experiment was designed to ascertain the nature of the mechanism that causes the difference in the number of spots on the varieties. In studying the nature of penetration, cotyledons of cucumbers and squash were used.

Seedlings were produced at temperatures from 20° to 28°C. in the greenhouse. Fifteen days after seeding, the cotyledons were cut off, and the lower surface was sprayed with conidial suspension and then put into a Petri dish on a sterilized moist filter paper. These were kept at a constant temperature of 24° or 28° C. The symptoms of the disease appeared on the inoculated leaves after 4-5 days at 24° C. and 3-4 days at 28° C. (Table 3).

Table 3. Number of lesions of cotyledons of cucumber and squash inoculated with *Colletotrichum lagenarium*[1]

Variety	Number of cotyledons tested	Susceptibility [2] Number of spots per unit area of leaf
Cucumber		
Tsuda-Sanjaku	11	2.8
Kwachu-Zairai	11	4.5
Taiwan-Kema	8	5.5
Nisshi-Aonaga	11	5.8
Su-Yo	11	6.4
Squash		
Shiro-Kikuza	6	0.2

1) With the exception of between Taiwan-Kema and Kwachu-Zairai, Nisshi-Aonaga, Su-Yo and between Nissi-Aonaga and Su-Yo, significant at less than 5 % level.
2) Area of cotyledons was calculated as an ellipse.

Cotyledons and leaves of cucumbers were equally susceptible to infection. Since cotyledons are more adaptable for experimental tests, they were used hereafter chiefly in experimental work.

In previous tests it was apparent that differences in the susceptibility among the varieties appeared to be primary stage of infections, particularly in the time of penetration. Therefore another test was made in which a heavy suspension of spores was used. On every cotyledon, ten drops of heavy or weak suspension of spores were placed and inoculated in a moist chamber at 28° C. One drop of weak suspension contained an average of a single conidium per field of microscope (× 150), whereas the thick suspension contained

about 89 conidia. In this test spots that originated from a single drop were recorded as one lesion, even though many minute spots initially appeared. From this test it was apparent that all the varieties were severely attacked by *C. lagenarium* when they were inoculated with heavy conidial suspensions. Varieties inoculated by weak spore suspensions differed greatly in number of lesions that developed. The data are in accord with the results given in Table 3.

It seems likely that the varietal difference in the susceptibility may be attributed to the amount of germination and initial infection, hence further tests were made in this areas. The germination tests of conidia were made in water drops which were gathered from the surface of cotyledons. Redistilled water was dropped on the cotyledons and the cotyledons were kept in a moist chamber for about 6 hours at 28° C. Then the drops on the cotyledons were gathered and transferred to a glass slide also kept at 28° C. The results indicate that the water drops gathered from the surface of cotyledons inhibited the germination of the conidia. However, there was no significant differences among the varieties (Table 4).

Table 4. Percentage of germination of conidia in water drops gathered from the surface of the cotyledons of cucumber and squash, respecitively

Variety	Number of conidia tested	Percentage germination	Percentage germination of conidia with appressoria [1]
Cucumber			
Tsuda-Sanjaku	1096	45.6	99.6
Su-Yo	1155	29.0	89.5
Squash			
Shiro-Kikuza	807	30.4	98.8
Redistilled water	1146	66.8	99.7

1) Based upon the germinated spores.

Studies were also made on germination of conidia in expressed sap of cotyledons and in a water drop containing a small piece of epidermis. Sap from each cotyledon was diluted three times with redistilled water. Almost all the conidia germinated and the germ tubes elongated vigorously without the formation of appressoria. Yoshioka and Naito [4] suggested that in watermelon the substances that inhibited the germination of conidia were located possible in the cuticle and epidermal cells. A similar study was made with cucumber. The lower epidermis of cotyledons in cucumber was stripped, and a small piece of this epidermis, about 4-6 mm^2, was floated in a drop on a glass slide. Data on the percentage of germination were based on an incubation period of 14 hours at 28° C.

Germination was greatly accelerated when pieces of epidermis were added to water drops. This also greatly stimulated appressorial formation that gave rise to infection hyphae.

The penetrating hypha that emerged from an appressorium invaded the epidermal cells and developed further into the tissues. Development of hyphae in the epidermal tissues of cotyledons of cucumber varieties were followed in considerable detail. The epidermis of cotyledons was peeled off and 48 hours after the inoculation the hyphae were stained with anilin blue acetic acid solution. Then the number of epidermal cells that were penetrated by an infection hyphae was determined. The results were based on 100-200 hyphae per

cotyledon involving one plant of each variety. There was no indication that varietal differences depended on number of cells that were invaded by the infection hypha.

The percentage of appressoria that gave rise to infection hyphae and that successfully invaded the epidermal cells was ascertained. In this test cotyledons from five plants per variety were used. Thirty hours after the inoculation the inoculated epidermis was peeled from the cotyledon and then stained as above (Table 5).

Table. 5. Percentage of penetrating hyphae from appressoria which invaded the epidermal cells of cotyledon. (30 hours after the inoculation)

Variety	Number of appressoria			Percentage of penetration
	Total	Per field of microscope	Giving rise to penetrating hyphae	
Cucumber				
Tsuda-Sanjaku	1623	4.1	336	20.7 [1]
Kwachu-Zairai	1132	2.6	313	27.7
Taiwan-Kema	1408	4.0	526	37.4
Nisshi-Aonaga	1542	3.7	634	41.1
Su-Yo	1428	3.3	624	43.7
Squash				
Shiro-Kikuza	1424	3.0	66	4.6

1) With the exception of between Taiwan-Kema and Nisshin-Aonaga, Su-Yo, and between Su-Yo and Nisshin-Aonaga, there is a significant difference at less than 5 per cent level.

The number of appressoria from which the infection hyphae arose was comparatively few. During this period they invaded only one epidermal cell. The number of appressoria formed was significantly different among the varieties of cucumber and squash. The invasion percentages given in Table 5 are listed again in Table 6 in order to compare with the inoculation test on cotyledons (Table 3). There was a positive correlation between the incidence of the disease and the percentage of invasion into epidermal cells (Table 6).

Table 6. Relation between the incidence of the disease on cotyledons and the percentage of penetrating hypha formation from appressoria.

	Squash variety	Cucumber varieties				
	Shiro-Kikuza	Tsuda-Sanjaku	Kwachu-Zairai	Taiwan-Kema	Nisshin-Aonaga	Su-Yo
Per cent invasion [1]	4.6	20.7	27.7	37.4	41.1	43.7
Relative value	11	47	63	85	94	100
Number of spots [2] per unit area of leaf	0.2	2.8	4.5	5.5	5.8	6.4
Relative value	3	45	70	86	90	100

1) Average of five cotyledons.
2) Average of six to eleven cotyledons.
$\gamma=0.996$, $\rho>0.916$, significant at 1 per cent level.

Thus the severity of this disease among varieties is associated with the difference in the number of successful penetrations of infection hyphae. These results tend to indicate that the resistance of a cucumber variety to invasion may be due to the structure of the epi-

dermal cell wall or the chemical nature of the cuticular layer, hence additional tests were made by wounding leaves.

The leaves were wounded by rubbing the leaf surface with fine particles of quartz sand. The wounding increased the relative number of spots both in resistant and susceptible varieties. Most of these openings were probably minute wounds in the epidermal cell wall. From the figures presented in Table 7, it is clear that the variety Su-Yo is more susceptible than Taiwan-Kema.

Table 7. Percentage of lesions on wounded and non-wounded leaves of cucumber when inoculated with *Colletotrichum lagenarium*.

Days elapsed after wounding	Su-Yo			Taiwan-Kema		
	Sound half leaf	Wounded half leaf	Per cent increase	Sound half leaf	Wounded half leaf	Per cent increase
0	[1] 2.7	3.9	44	0.4	1.8	350
1	2.5	4.0	60	0.6	2.0	233
4	1.5	3.0	100	0.3	1.2	300

1) Number of lesions per unit area (cm^2) of leaf, average of 3-4 leaves. All leaves were inoculated simultaneously.

Discussion

The inoculation tests verified previous work, namely that marked differences in susceptibility to the anthracnose fungus exists among species of the Cucurbitaceae. All the squash varieties tested were highly resistant whereas there were striking variations in susceptibility among varieties of cultivated cucumbers. The conidia of *C. lagenarium* germinated and developed vigorously in both the expressed sap of cotyledons and in the water drops which contained small pieces of epidermis of cotyledons of resistant and susceptible cucumber varieties. This indicates that the mechanism giving rise to the varietal difference in susceptibility is not related to germination and the production of appressoria. The successful penetration of the host was definitely associated with the variation in susceptibility. In fact a positive correlation of $\gamma=0.996$ existed between a number of spots per leaf and degree of susceptibility. Moreover, the wounding of the leaf surface greatly increases the percentages of spots in both resistant and susceptible varieties of cucumber, further suggesting that resistance and susceptibility are associated with the number of successful penetrations of infection hyphae.

Summary

The inoculation experiments with *C. lagenarium* on mature leaves involving varieties and species of cucurbitaceae indicated that all plants were invaded by the pathogen. Al varieties of squashes tested were highly resistant to *C. lagenarium*, whereas some varieties of cucumbers (Tsuda-Sanjaku, Sekino No. 2, and Wild cucumber) were resistant, others, such as Su-Yo, and Kwachu-Zairai were susceptible.

The varietal difference in the susceptibility also was apparent by inoculating cotyledons and mature leaves. Although the cotyledons of Shiro-Kikuza (squash) were affected slightly, the mature leaves of this variety were not infected. Lesions developed at appoximately the same rate in all varieties of cucumbers. After the hyphae are established in the host, they develop equally well in resistant and susceptible varieties.

Sap extract of cotyledons of cucumber varieties accelerated the germination of conidia. The conidia of *C. lagenarium* germinated well in the water drops gathered from cotyledons of resistant and susceptible varieties that had previously been moistened with distilled water. Even when the drops on cotyledons of squash were highly resistant to anthracnose, the conidia germinated well and formed appressoria.

The percentage of infection of hypha that formed from the appressoria on cotyledons differed significantly among the cucumber varieties. Also, there was a high correlation between the degree of the infection hypha formed and the number of spots per unit of area of leaf. This presumably accounts for the difference in the susceptibility among varieties of cucumber. The susceptibility also may be related to the character of epidermal cell wall.

Literatures cited

1. Gardner, M. W.: U. S. Dept. Agr. Bull. 727: 1-68, 1918.
2. Iwata, I.: Agr. and Horticult., Tokyo, 22(6): 309-310, 1947.
3. Kawai, I.: Ibid., 28(5): 638-640, 1953.
4. Yoshioka, A. and K. Naito: Ann. Phytopath. Soc. Japan, 18: 90-91, 1953.

Shokubutsu Byogai Kenkyu, Kyoto, 6(3): 104~108, 1959.

Biochemical studies on *Cochliobolus miyabeanus*

(S. Ito et Kurib.) Drechsler ex Dastur

II. Enzymes concerning amino acid utilization

(Part 1.) On the amino acid oxidase

Hachiro Oku*

Introduction

In the previous paper [3], the author reported that *Cochliobolus miyabeanus* utilized various amino acids as a sole source of carbon or nitrogen, and produced a few keto-acids, such as pyruvate, α-ketoglutarate, and oxalacetate, in the culture medium. To clarify the amino acid metabolism of this fungus, the enzymes concerning the amino acid utilization should be investigated. Recently, Otani[4] suggested that *l*-amino acid oxidase may play an important role in the amino acid utilization of *Piricuralia oryzae*. Therefore, an experiment was carried out to test amino acid oxidase activity of *C. miyabeanus*. Unexpectedly, *l*-amino acid oxidase activity was not detected, while high activity of *d*-amino acid oxidase was found in this fungus. Consequently, the characteristics of *d*-amino acid oxidase were tested.

Methods

1) Preparation of enzyme. Acetone dried mycelium was prepared. *Cochliobolus miyabeanus*, was raised in a shaking flask containing 100 ml. of the modified Czapek medium**. After 64 hours' shake culture at 26° C., mycelia were harvested and washed with distilled water. Then, they were put into acetone at −30° C. in dry ice-acetone bath and beaten for 3 min. in Waring blendor cup. Then, they were washed with acetone at 0~5° C, and dried in a vacuum desiccator at room temperature. After drying, the mycelium was ground into fine powder.

2) Measurement of enzyme activity. Using Warburg manometric technique oxygen consumption was determined at 30° C. 50mg of acetone-dried mycelium and M/15 phosphate buffer were put into main compartment and 0.5ml. of M/10 *l*-or M/5 *dl*-amino acid into side arm. KOH solution was poured into the well. The atmosphere was air.

In all experiments the small autorespiration was corrected by placing enzyme suspension in the thermobarometer vessel.

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** Medium. ——— glucose 20g, peptone 5g, K_2HPO_4 1g, KCl 0.5g, $MgSO_4 \cdot 7aq$ 0.5g, $FeSO_4 \cdot 7aq$ 0.01g, tap water 1000 ml. pH 6.8.

Results

1) Enzyme specificity and stoichiometric relations. A rapid oxidation was observed by the addition of various *dl*-amino acids. When *l*-amino acid was substituted for the racemic mixture, no oxidation occurred. Addition of FAD and FMN, the coenzyme of amino acid oxidase, did not show any effect.

This fact suggests that either no activity of *l*-amino acid oxidase was found or the enzyme was inactivated during the preparation procedure. Therefore, the living mycelium was substituted for the acetone powder, and the ability to oxidize *l*-amino acid was tested at pH 6.0, 7.0, and 8.0. No oxidation, however, occurred. Fermentation broth had no activity of *l*-amino acid oxidase.

Table 1. Rate of oxidation of amino acids by the amino acid oxidase in acetone dried mycelium of *C. miyabeanus*

Amino acid	O_2 uptake (μl)	
	30 min.	60 min.
dl-Methionine	65	127
dl-Alanine	59	118
dl-Valine	55	107
dl-Leucine	49	100
dl-Phenylalanine	47	93
dl-Isoleucine	45	88
dl-Aspartic acid (Na)	27	54
dl-Tryptophane	22	41
dl-Serine	14	37
dl-Lysine-HCl	11	19
dl-Glutamic acid (Na)	7	15
dl-Proline	5	12
dl-Tyrosine	3	8
dl-Histidine-HCl	2	4
l-Methionine	0	0
l-Alanine	0	0
l-Glutamic acid (Na)	0	0
l-Aspartic acid (Na)	0	0
l-Proline	0	0
l-Alanine+FAD	0	0
l-Alanine+FMN	0	0

As shown in Table 1, *dl*-methionine is the most rapidly oxidizable substrate by the enzyme of *C. miyabeanus*, and *dl*-alanine, *dl*-valine, *dl*-leucine are also good substrates. Glutamic acid, proline, tyrosine, and histidine were oxidized slowly.

2) Effect of enzyme concentration. Between enzyme concentration and oxygen consumption with *dl*-methionine as a substrate, liner correlation was found (Fig. 1).

3) Optimum pH of *d*-amino acid oxidase. The effect of pH on the enzyme activity was determined with *dl*-methionine as a substrate. Britton and Robinson's wide range buffer was used. A marked optimum was observed at pH 10.5 (Fig. 2).

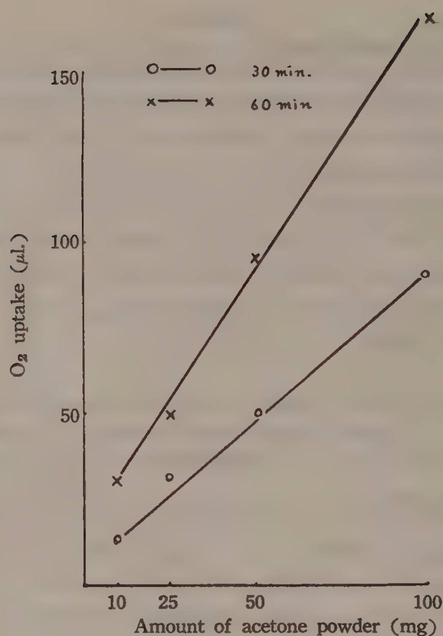


Fig. 1. Effect of enzyme concentration on oxygen consumption

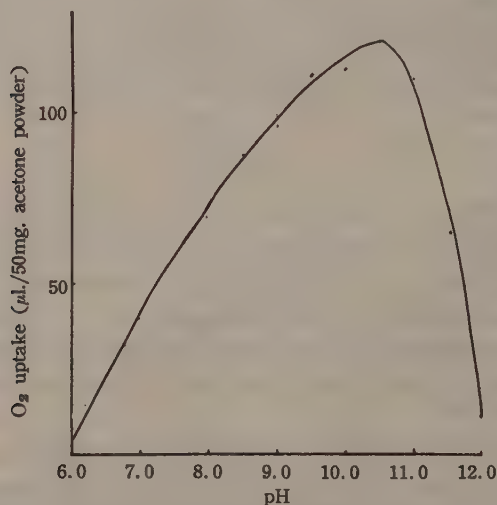


Fig. 2. Effect of pH on the *d*-amino acid oxidase of *C. miyabeanus* with *dl*-methionine as a substrate.

4) Inhibitors of *d*-amino acid oxidase. The enzyme was not inhibited either by 0.01 M KCN, or 0.001 M iodoacetate. Benzoate (0.01 M) inhibited about 40 % of the activity. 2, 4-Dinitrophenol inhibited completely at a concentration of 0.01 M.

5) Identification of keto acid. Pyruvic acid, the keto acid analogue of alanine, was found to be an oxidation product of *dl*-alanine by the enzyme. It was identified from the

Table 2. Effect of inhibitors on the *d*-amino acid oxidase of *C. miyabeanus* with *dl*-methionine as a substrate.

Inhibitor	Conc. (M)	% inhibition
$(NH_4)_2SO_4$	1.0	68
	0.1	0
Benzoic acid	0.1	100
	0.01	43
p-Aminobenzoic acid	0.1	100
	0.01	30
p-Aminosalicylic acid	.1	75
	0.01	13
2,4-Dinitrophenol	0.01	100
	0.001	46
$CuSO_4 \cdot 5H_2O$	0.1	100
	0.01	0
KCN	0.01	0
	0.001	0
CH_2ICOOH	0.01	66
	0.001	0

reaction mixture in the form of 2,4-dinitrophenylhydrazone by paper chromatography using n-butanol: ethanol: 0.5 N NH_4OH (70 : 10 : 20) as a solvent. The phenylhydrazone of pyruvic acid gave 2 spots, and their R_f values were 0.41 and 0.61.

Discussion

Since *C. miyabeanus*, when cultured in the above described medium, had no activity of *l*-amino acid oxidase, other enzymes might play a role in the *l*-amino acid utilization. However, high activity of *d*-amino acid oxidase had been detected in *C. miyabeanus*. The function of this enzyme in the metabolism is unknown.

d-amino acid oxidase of *C. miyabeanus* differs from enyzmes of other sources in several characters. Horowitz[2] reported that drying the mycelium of *Neurospora* with acetone and ether resulted in inactivation of enzyme. But in the case of *C. miyabeanus* this treatment did not affect the activity. *d*-amino acid oxidase of *C. miyabeanus* differed markedly from the enzymes of other sources in the optimum pH. The former has the optimum at pH 10.5, but the latter at 8.0~9.0.

Summary

1. The activity of *l*-amino acid oxidase was not detected, while high activity of *d*-amino acid oxidase was found in *Cochliobolus miyabeanus*.

2. The characteristics of *d*-amino acid oxidase were investigated with the acetone dried mycelium.

3. The optimum pH value of the enzyme was found to be 10.5.

4. The enzyme was not destroyed by drying. 2,4-Dinitrophenol inhibited the activity

of the enzyme completely at the concentration of 0.01 M, but not by potassium cyanide. Benzoate did not inhibited significantly.

5. *d*-Form of the following amino acids were rapidly oxidized: methionine, alanine, valine, leucine, phenylalanine, but the following was slowly oxidized: lysine, glutamic acid, proline, tryptophan, and histidine.

Literatures cited

1. Dixon, M.: Enzymologia, 5: 198, 1938.
2. Horowitz, N. H.: J. Biol. Chem. 154: 141, 1947.
3. Oku, H.: Ann. Rep. Takamine Research Lab. 10: 134, 1958.
4. Otani, Y.: Ann. Phytopath. Soc. Japan, 18: 159, 1954.

植物病害研究 (Shokubutsu Byogai Kenkyu, Kyoto), 6(3) : 109〜113, 1959.

秋落稲の胡麻葉枯病罹病性に関する研究

第5報　培地の炭素源，アミノ酸量ならびにC-N率が病原菌の殺生酵素分泌に及ぼす影響

浅　田　泰　次*

Yasuji Asada: Studies on the susceptibility of akiochi (autumn-declined) rice plants to *Helminthosporium* blight. (V). On the influence of the carbon sources, the amount of amino acids and C-N ratio upon the secretion of the perthophytic enzymes of *Cochliobolus miyabeanus*.

1. 緒　　言

筆者[5]は先に秋落稲は正常稲にくらべて蛋白態窒素が少なく，可溶性窒素が多い事実をのべた．いまこの結果から秋落稲及び正常稲の可溶性窒素対蛋白態窒素の比率をみると，前者では 0.30，後者では 0.24 となる．この値の大きいことは蛋白の合成が妨げられていることを示しているが，可溶性糖対可溶性窒素の比率すなわちC-N率をみると，秋落稲では13.94，正常稲では12.30である．ところで稲胡麻葉枯病菌の殺生作用は，まず第1段階としてそれが分泌する cellulase, xylanase, pectic enzyme のような体外酵素によると考えられるが[4]，前述のような窒素比とかC-N率などの相違が酵素の作用場での体外酵素分泌量に差を生ぜしめるものと予想される．すなわち秋落稲において病斑拡大の著しいことは，病原菌が秋落稲体内に発育した場合酵素の分泌が多く，従つて殺生作用も強力に発揮されるのではないかと思われ，さらに寄主自体の代謝活性が弱いことにもよるものと想像される．しかして寄主体内に栄養源が多いことは，それだけ病原菌の発育が良好となり，病斑が大きくなるものと考えられるが，本菌のような殺生菌の場合には，病原菌が発育するためには寄主細胞を殺す必要があり，病斑が大きくなることは殺生が盛んであることを意味するであろう．従つてこのような病原菌の殺生能力に寄主体内養分の質的あるいは量的差異が影響するものと考えた方が妥当ではないかと思われる．しかし正常稲ならびに秋落稲における病斑拡大度の差は，病原菌側の病原性と寄主側の抵抗反応との差として肉眼的に観察されることは今さらのべるまでもない．本報告においては病原菌側の立場から，その病原性（殺生作用）の強弱を培地のアミノ酸量，炭素源及びC-N率をかえて比較し，さらに正常稲及び秋落稲煎汁中の病原菌の cellulase, xylanase, pectic enzyme などの活性度を測定して，前述の推定との平行関係を調べた．

本稿を草するに当り，終始御指導御鞭撻を頂いている京都大学赤井重恭教授，本学吉井　啓教授に深く感謝の意を表する．また実験に多くの援助を頂いた木曽　皓助手，徳永省三，加田隆稔の諸氏に厚く御礼申し上げたい．

2. 実験材料ならびに方法

供試菌は当研究室保存，H-1 号株（栃内・坂本の系統群Ⅲに相当する）であり，培地は筆者の創製したもの[3]を基準として，そのC-N率及びアミノ酸量を第1表の如く変えたものである．なお炭素源には基準培地の蔗糖と同量の xylose, xylan, starch, pectin**などを用いた．秋落稲は既報[6]の如く水耕栽培した稲を一定期間可溶性澱粉で処理したものであるが，それらの生葉 10g を 100ml の蒸溜水で 100°C で，1時間浸出して蔗糖3%を加えたものをも培地として用いた．培地は 50ml 容三角フラスコに 10ml 宛分注し，0.5 気圧15分間滅菌後供試菌（1%蔗糖加用馬鈴薯煎汁寒天培地に 28°C．で，20日間培養のもの）を移植して，28°C．下に10日間発育せしめた***．培養後菌体を沪紙で沪過し，沪液を酵素液として用い，菌体は恒量になるまで乾燥して秤量した．pH 測定には東洋沪紙 pH 試験紙を用いた．しかして試験管に1%基質液 2ml, McIlvaine 緩衝液 4 ml，酵素液 4ml, toluene 1ml を加え，それらを 30°C．下に24時間作用せしめた後，酵素の力値測定を行つたが，それには基質の分解による還元力を dinitrosalycylic acid 法[8]

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* 愛媛大学農学部植物病理学研究室　昭和31年11月8日受理

** xylan は日比野[2]の方法により自製，他は片山化学社製品．この場合溶質のモル濃度を同じにすることも考えられるが，xylan, starch, pectin などは正確な分子量がわからないので単に百分率濃度を同一にした．

*** 培養沪液内の酵素の活性は培養10日前後が最も著しいが，その時期は発育対数期に相当する．

により光電比色計で比色定量した．なお基質は pectin, xylan 及び cellulose である．しかして酵素液中には最初から残糖その他の還元性物質が存在するから，各試験区とも対照として酵素液を100°C．で30分加熱して不活性化し，容量を補正して酵素液と同様に作用せしめてえた数値を試験区の測定値から控除した．結果は glucose の還元力に換算して比較検討したが，2回反覆した内の1例（flask 3個の平均値）を示した．

第1表　供試培地中の窒素源ならびに炭素源量
Amount of N- and C-sources in the basal medium* used in this experiment.

Chemicals	Media													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
KNO_3 g	54.5	40	30	10	5.5	1.2	0.6	—	—	—	—	—	—	—
Na-glutamate	—	—	—	—	—	—	—	1	2	3	5	10	20	40
Sucrose	5.5	20	30	50	54.5	58.8	59.4	50	50	50	50	50	50	50
C-N ratio**	0.1	0.5	1	5	10	50	100	—	—	—	—	—	—	—

* Basal medium: KH_2PO_4 5g, $MgSO_4 \cdot 7aq$ 2.5g, $ZnCl_2$ 15mg, $CaCl_2 \cdot 6aq$ 5mg, $FeSO_4 \cdot 7aq$ 1.5mg, $CuSO_4 \cdot 5aq$ 0.15mg, $MnSO_4 \cdot 7aq$ 0.11mg, Thiamine 1.25mg, Biotin 12.5γ in 1*l* distilled water. pH 5.7.

** Ratio of sucrose to potassium nitrate.

3. 実験結果ならびに考察

(1) アミノ酸量と菌体発育ならびに殺生酵素分泌量　病原菌の栄養となる寄主体内の窒素は可溶性窒素であると思われるが，培地中のアミノ酸の多少は病原菌の酵素活性の強弱，すなわち病原菌の酵素分泌量にほぼ比例的に影響する(第2表)．寄主体内においてもこれと同じような現象が起りうるものとするならば，可溶性窒素が多いほど病原菌の殺生に関与する酵素（殺生酵素と呼ぶ）の分泌が盛んになり，菌体発育もまた良好となるものと思われ，このことはまた病斑拡大を助長する1要因となるものと思われる．寄主体侵入直後の病原菌は，当然菌自体が最初から持つていた energy によつて酵素を分泌し，殺生を行うであろう．しかしそれ以後においては死滅した寄主体内の養分の多少が菌の発育に影響し，ひいては殺生酵素の分泌にも影響する．培地Nでは培養沪液の pH がかなりアルカリ性になるが，これは Na-glutamate の分解吸収に伴い Na 塩が培地にのこるためと考えられる．また培地Lのアミノ酸量は通常用いられる Richards 培地のKNO_3量に相当し，本実験の場合供試菌の発育は 141mg である．しかるにアミノ酸をKNO_3に代えた場合には僅かに 71mg を示すに過ぎず，本菌は無機態窒素よりも有機態窒素の方をよりよく利用するようである．

第2表　稲胡麻葉枯病菌の発育ならびに殺生酵素分泌に及ぼす培地内アミノ酸量の影響
Influence of the amount of amino acid on the mycelial growth and on the activity of perthophytic enzymes in the culture filtrate of *Cochliobolus miyabeanus.*

Experiments	Media*						
	H	I	J	K	L	M	N
Dry weight of mycelium mg	38	58	59	99	141	187	190
Final pH of filtrate	5.4	5.8	6.2	6.6	7.4	8.0	8.2
Cellulase**	0	2.3	4.6	9.1	4.6	4.6	0
Xylanase**	0	6.8	9.1	9.1	22.8	27.3	5.0
Pectic enzyme**	0	0	2.3	9.1	9.1	36.4	3.6

* Amount of amino acid in the media is presented in Table 1.

** Activity is shown by the reducing power of glucose.

(2) 炭素源と菌体発育ならびに殺生酵素分泌量　稲胡麻葉枯病菌は寄主稲の細胞間隙を伸長し[1,15] pectic enzyme, cellulase, xylanase などによつて細胞膜を局所的に分解し栄養を吸収しているものの如く考えられる[4]．従つて本菌の利用する稲体内の炭素源としては starch, glucose, xylan, xylose, 各種有機酸, pectin などを考えればよい．筆者はこれらの炭素源を用いて病原菌を培養し，その発育ならびに殺生酵素の分泌に及ぼす影響を調査した．結果は第3表の通りであつて，xylan や starch を炭素源とすると xylanase や pectic enzymeが比較的多量に分泌される．菌体発育の順位は starch>xylan>xylose>glucose>pectin となるが，酵素の分泌は cellulase では glucose>starch>xylan>xylose>pectin, xylanase では xylan=starch>glucose>

xylose>pectin, pectic enzyme では pectin>xylan=starch=xylose>glucose の順となる．以上のことから寄主稲体内において病原菌が利用しうる炭素源は，恐らく glucose や xylose のような単糖でなく，それらの polymer である starch, xylan などではないかと思われ，自然に近い状態としてはこれらの polymer を炭素源とする方がよく，殺生酵素もまた盛んに分泌されるものと考えられる．禾本科植物の細胞間物質（中葉）は殆んど pectin 及びその誘導体と考えられる[14]．従つて pectin そのものもまた病原菌の炭素源として幾分役立つものと思われる（第3表）．田

第3表　稲胡麻葉枯病菌の発育ならびに殺生酵素分泌に及ぼす各種炭素源の影響

Influence of carbon sources on the mycelial growth of *Cochliobolus miyabeanus* and on the activity of the perthophytic enzymes secreted in the culture filtrate.

Experiments	C-sources				
	xylan	xylose	starch	glucose	pectin
Dry weight of mycelium mg	128	116	177	85	19
Final pH of filtrate	7.0	6.8	7.0	6.4	3.0
Cellulase *	16.2	8.1	32.4	48.6	0
Xylanase *	40.5	8.1	40.5	32.4	0
Pectic enzyme *	16.2	16.2	16.2	8.1	64.8

* Activity is shown by the reducing power of glucose.

中[12]によれば本菌の炭素源には starch よりも xylose や glucose が良好であつて，第3表の結果とは一致しない．このことは恐らく筆者の実験では培地の百分率濃度を同じにしたために*，各培地中のモル濃度が同じでない為であると思われるが，さらに菌の系統の相違，あるいは基準培地の組成，特に生育因子の有無，培養条件などによるものと考えられる．玉利[10]は稲熱病菌がその系統によつて発育に対する biotin の必要性や炭素源の要求が異ると述べているが，本菌の場合にも同様なことがあるかも知れない．本病罹病稲の解剖学的所見[1]によれば本菌は寄主の細胞間隙を伸長するが，本菌の発育に必要な窒素源を始め微量要素である biotin, thiamine, Zn, Fe, Mn など[3]は，細胞間物質中には存在しないものと考えられる．従つて細胞内養分の吸収を可能にするために，細胞膜の一部分解が菌によつて行われるのではないかと思われる．さらに本病々斑には一般に崩壊部が認められないから，全体的な細胞膜の分解利用は考えられず，局部的分解によつて細胞内養分の吸収が行われるのでないかと考えられる．しかし一方細胞膜にはミセル空隙があると云われているから，溶質分子の拡散は菌絲が細胞膜に接着するだけで可能であつて，特に分解を必要としないかも知れない．しかし細胞の半透性はそれが既に死んでいる以上なくなつていると思われるから，やはり局所的な分解を考えるのが妥当のように思われる．それでは既に死んだ細胞に，前述の cellulase や xylanase, pectic enzyme などが作用することは殺生とは無関係の如く考えられるが，上記の酵素が殺生に関係するという意味は，それらの酵素によつて中葉が分解され，細胞相互間の動的平衡が破壊されることが殺生ではないかと考える．高橋[11]は微針で1細胞を物理的に傷つけても，その周りの細胞まで褐変壊死を起すとのべている．このことは細胞相互間が相互的動的平衡状態にあつて，その平衡の破壊によつても細胞死が起ることを意味するのでなかろうか．いずれにせよこのようにして吸収利用された starch, xylan など

第4表　稲胡麻葉枯病菌の発育ならびに殺生酵素分泌に及ぼす C-N 率の影響

Influence of C-N ratio in media on the mycelial growth of *Cochliobolus miyaneanus* and on the activity of the perthophytic enzymes secreted in the culture filtrate.

Experiments	Media*						
	A	B	C	D	E	F	G
C-N ratio	0.1	0.5	1	5	10	50	100
Dry weight of mycelium mg	17	93	95	122	104	81	52
Final pH of filtrate	5.8	6.4	6.8	7.0	7.2	6.0	5.2
Cellulase **	1.6	2.6	—	8.4	17.6	8.8	0
Xylanase **	1.2	1.4	2.6	26.4	13.2	11.4	1.5
Pectic enzyme	3.6	4.2	1.9	30.8	17.6	17.6	0

* The composition of media is presented in Table 1, and the medium D is the modified Richards' medium with minor elements.

** Activity is shown by the reducing power of glucose.

* 絲状菌発育の炭素源を決める場合，百分率濃度，モル濃度あるいは炭素原子量（この場合は供試薬剤の分子量がわかつている必要がある)のいずれを同一にすればよいかは種々論議のあるところである．いま，溶媒 1L 中に 50g の glucose と sucrose を与えたとすると，両者の百分率濃度は共に5％，モル濃度では前者は0.14 mol，後者は0.28 mol で，炭素原子自体の量は前者は 20g，後者は 21g である．しかし菌が糖を吸収利用する場合は炭素原子としてでなく，glucose ならば glucose 分子として吸収するのではないかと思われるので，やはり分子量自体を同じにして比較した方が適切ではないかと思われる．

はまた本菌が次の中葉その他を分解するための殺生酵素分泌の energy 源となつているものであろう．

(3) C-N率と菌体発育ならびに殺生酵素分泌量 秋落稲のC-N率は正常稲よりやや高いので，C-N率と菌体発育ならびに酵素分泌量との関係を推察するため，まず培地の KNO_3 及び sucrose の添加量をかえて実験した．最初 sucrose 量を一定(50g/l)にして，KNO_3 の添加量によつてC-N率を変化せしめたが，例えば C/N=1 の場合は培地に加える全成分量が 1l 当り 100g をこえ，培地の滲透圧はかなり大きくなる．従つて sucrose 及び KNO_3 の和を一定にしてC-N率をかえ，実験を行つた（第1表参照）．第4表に示した如く，C-N率（sucrose 量と KNO_3 量の比）が5～10の場合，菌体発育量ならびに酵素分泌量が多い．しかし寄主体内のC-N率とは可溶性糖と可溶性窒素との比であり，供試培地では sucrose と KNO_3 との比であるから，秋落稲のC-N率が正常稲のそれよりも大きいからといつて，直ちに殺生酵素分泌量も多いとは云い難い．また両者の成分組成の相違からしても断定は困難である．従つて本実験の結果からは，培地のC-N率の違いが病原菌の発育ならびに酵素分泌に差を生ぜしめるとしかいいえない．

(4) 稲葉煎汁培地での菌体発育ならびに殺生酵素分泌量

前項までの観察は，全く合成培地を用いたものであつたが，寄主葉煎汁を培地にした場合の結果を知るために，正常稲葉ならびに秋落稲葉煎汁を培地として，病原菌の発育ならびに酵素分泌量を比較した．しかしこれも葉の煎汁であるから，全く in vitro の試験に過ぎない．しかし in vivo において殺生された細胞の内容物が栄養源となつていると思われるから，煎汁を作るための熱処理による変質を考慮外とすれば，in vivo とはまつたくかけはなれた無意味な試験とは思われない．結果は第5表の通りであつて，秋落稲葉煎汁を培地とする方が菌体の発育もよく，酵素の分泌もまた盛んである．

以上の4項から推察すると，秋落稲においては侵入した病原菌の殺生作用がより発揮し易い状態にある如く思われ，このことが大型病斑の現れる1要因ではないかとも考えられる．稲熱病に罹り易い稲においても可溶性窒素の蓄積が著しいが[7,13]，稲熱病菌は殺生菌であるから，その殺生原因の一つである α-picolinic acid や piricularin の分泌[9]は，菌が可溶性窒素を多く吸収利用すればする程著しく，殺生作用もまた盛んとなつて病斑の拡大を来すものではなかろうか．

第5表 稲胡麻葉枯病菌の発育ならびに殺生酵素分泌に及ぼす正常稲葉及び秋落稲葉煎汁液の影響

Influence of the decoction of normal and akiochi rice leaves on the mycelial growth of *Cochliobolus miyabeanus* and on the activity of the perthophytic enzymes secreted in the culture filtrate.

Decoction	Dry weight of mycelium	Final pH of filtrate	Cellulase	Xylanase	Pectic enzyme
Normal leaves	mg 53	6.2	* 1.9	1.9	3.9
Akiochi leaves	64	6.8	7.8	3.9	5.8

* see Table 4.

4. 摘要

寄主体内の化学成分から寄主の罹病性を推察するには，まず寄主成分の質あるいは量的差異が侵入した病原菌の殺生作用にいかに影響するかを確める必要があると思われ，このような影響が病斑拡大に差を生ずる1要因とも考えられる．一般に秋落稲は正常稲にくらべて可溶性窒素が多く，C-N率（可溶性糖対可溶性窒素の比）もまた大である．従つてこれらの殺生作用に及ぼす影響を知るために，まず合成培地内のアミノ酸量及びC-N率を変えて，胡麻葉枯病菌の殺生酵素すなわち cellulase, xylanase, pectic enzymeなどの分泌量ならびに菌体発育に及ぼす影響を調べた．この結果，C-N率(sucrose と KNO_3の比)が5前後のばあいならびにアミノ酸量の多いときには酵素の分泌量も多く，菌体発育もまた良好である．しかして培地の炭素源は百分率濃度で比較した時は glucose よりも xylan や starch の方が供試菌の菌体発育ならびに酵素分泌に好結果をもたらしている．しかして秋落稲葉煎汁を培地にしたばあいには，正常稲葉煎汁培地にくらべて菌絲発育ならびに酵素分泌量が著しい．すなわち秋落稲は可溶性窒素の量も多く，殺生され易い状態にあるものの如く，病原菌の侵害せられた後においても正常稲よりも殺生酵素の分泌が多いのではないかと推察される．本病罹病性を病原菌側のみからみたばあい，このような関係が大型病斑の現れる一要因をなしているのでないかと思われる．

引用文献

1. 赤井重恭：禾穀類の病害に関する研究（謄写印刷），5-7，1945.
2. 荒木長次：化学実験学 II，天然物取扱法 III，東京，p. 366，1943.
3. 浅田泰次：日植病報，21：68-70，1956.
4. ————：同上，21：191-193，1956.
5. ————：同上，22：103-106，1957.
6. ————：植物防疫，10：464-466，1956.
7. 大谷吉雄：日植病報，16：97-102，1952.
8. 大槻虎男：標準生化学実験，東京，p. 18，1953.

9. 玉利勤治郎：科学，25：18-23，1954.
10. ――――：植物防疫，11：233-239，1957.
11. 高橋喜夫：栃内，福士両教授還歴記念論文集，245-248，1955.
12. 田中寛康：植物病害研究，5：165-170，1956.
13. 田中正三：化学，7：678-688，1952.
14. 山田　登・丸尾文治：ボナー植物生化学，東京，p. 102，1954.
15. 吉井　甫：日植病報，9：170-172，1939.

Summary

As a step in indicating the desease-susceptibility in rice plants, it is necessary to clarify the influence of the qualitative and quantitative difference in the chemical components of the host variety upon the perthophytic activity of the invading pathogenic fungus. This may be a key for clarifying the mechanism of enlargement of spotted area of leaves. Akiochi rice plants, i. e., physiologically declined rice plants in autumn, contain more soluble nitrogen than the normal ones. The C-N ratio in these plants is usually high. The writer made an in vitro test on the influence of amino acids, carbon sources and C-N ratio upon the secretion of the extracellular perthophytic enzymes (cellulase, xylanase and pectic enzyme) of the *Helminthosporium* blight fungus of rice plants. The mycelial growth was vigorous showing the high activity of perthophytic enzymes, when the amino acids were in abundance in the media and the C-N ratio (the ratio between the amounts of sucrose and potassium nitrate) was about 5. When xylan or starch was used as a carbon source, the secretion of xylanase and pectic enzyme was more promoted than when glucose was used. When the decoction of leaves of akiochi rice plants was used as a culture medium, the secretion of the perthophytic enzymes of this fungus was more active than that of the normal ones. From these tests it is very probable that since the akiochi rice plants contain more available soluble nitrogen than the normal plants. They are in a condition more readily attacked by the causal fungus and that they are also more tolerant to the secretion of fungus enzymes.

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植物病害研究 (Shokubutsu Byogai Kenkyu, Kyoto), 6(3): 114~116, 1959.

秋落稲の胡麻葉枯病罹病性に関する研究

第8報　病原菌の蓚酸分泌とペクチン分解

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Yasuji ASADA: Studies on the susceptibility of akiochi (autumn-declined) rice plants to *Helminthosporium* blight. (VIII). Production of oxalic acid by the pathogenic fungus and decomposition of pectin.

Wehmer[6] は絲状菌が糖類から有機酸を生成することを見出し，Nordhansen[8] は病原菌の分泌する蓚酸が寄主侵入に与る一要因であると述べている．樋浦，黒田，永田ら[9,10,11] も白絹病菌が蓚酸を分泌することを認めたが，殺生作用との関連には疑問を持つている．絲状菌の蓚酸代謝については，Foster[6] の著書に詳しく述べられている．しかし病理学的立場からみれば，病原菌の蓚酸分泌によつて寄主がどのような影響を受けるかが問題であつて，殺生菌の殺生機作に対して蓚酸説と酵素説が古くから対立している[12]．筆者[2,4]は，本菌の殺生機作を，菌絲の物理的な傷作用とそれが分泌する pectic enzyme, cellulase, xylanase などによる寄主組織間の動的平衡の破壊とに求めているが，本菌が蓚酸を分泌すればその寄生性から考えて，中葉成分である pectin が分解する可能性があり，きらに前報[2]で述べた pectic enzyme の活性が培養初期にみられないのは，基質の分解による還元力をもつて活性を測定したからではないかと考え，この点についても補遺試験を行つた．

本稿を草するに当り，終始御指導御鞭撻を頂いている京都大学赤井重恭先生，本学吉井　啓先生，実験に当り多大の援助を頂いた木曽　皓，徳永省三の諸氏に厚く御礼申し上げたい．

1.　実験材料ならびに方法

供試菌は研究室保存H-1号株，培地は筆者の創製したものを用い，50ml 容三角フラスコに10ml 宛分注して，28°C 下に5乃至25日間培養した．蓚酸による pectin 分解ならびに pectic enzyme (depolymerase) 活性の測定は粘度降下法によつた．すなわち Ostwald 粘度計を用い，1% pectin 液 5ml に酵素液として培養沪液 5ml あるいは所定濃度の蓚酸液を加え，30°C の定温器に入れて2時間後の粘度降下を30°C の恒温槽中でストップウオッチで測定し Pectin 分解度をみた．また沪液を100°C 下で，30分加熱して酵素を失活せしめたもの 5ml を加えて測定し，これを酵素作用以外の要素による分解度とした．対照として蒸溜水 5ml を加えて測定し，これより先の各試験区の数値の差を比較検討した．沪液中の蓚酸を確認するにはペーパークロマイトグラフィを行つた．すなわち沪液を水蒸気蒸溜し，残液を濃縮したもの，あるいは硫酸酸性でソックスレー抽出器で3日間エーテル抽出したものを試料とし，東洋沪紙 No. 50 を用いて n-ブタノール，酢酸，水（4：1：2）を溶媒として上昇法で一次展開した．展開後0.1% BCGを噴霧して発色せしめた．対照には5%純蓚酸を滴下して展開し比較した．蓚酸の基準 Rf は0.32～0.34である．

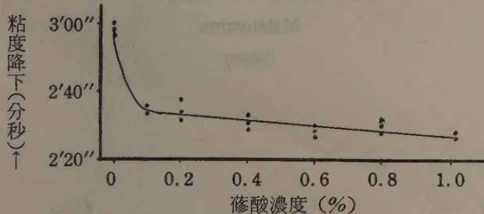
2.　実験結果ならびに考察

(1) 蓚酸分泌の確認　飯田，古山，綾[13]は本菌が蟻酸，乳酸，酒石酒，蓚酸，クエン酸，燐酸を生成することを報告したが，この中で筆者は蓚酸が培地内に生成されることをペーパークロコトグラフィで確認した．培養5日後の沪液にも既に蓚酸の生成が認められ，10日乃至20日後はもち論，さらに培養日数が経過すると培地内に結晶として析出することがある．このように培養に伴つて生成蓚酸量は増加するものと推定されるが，培養初期には培地内に残糖その他が存在するので，これらを除かないと直接 $KMnO_4$ で滴定出来ない．従つて定量的に量的変動をみることはできなかつた．他の有機酸については，沪液の濃縮程度によつて spot の出現が一定でなく，蓚酸と比べてその生成量は非常に少ないと考えられる．また飯田ら[13]の分析は試料として培養沪液と菌体が一緒になつているから，例えば乳酸の如きは菌体内成分ではないかと思われる．蓚酸の precursor が glucose であることは間違いないが，永田，林[14]は白絹病菌の蓚酸は TCA cycle をえて生成されること，さらにコハク酸→酢酸→グリコール酸→グリオキシ酸の経路も存在すると推定している．島薗[14]は *Coriolus hirsutus*

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の蓚酸を蟻酸とCO_2に分解する酵素が，モノヨード酢酸によつて阻害されると述べているが，永田，林[14]は10^{-3}Mのモノヨード酢酸を培地に加えると，蓚酸が著しく蓄積すると述べている．こうした事実を考え合せると，蓚酸は菌の分泌したものであり，培地内で菌が繁殖するために2次的に生成されたものではないと考えられる．一般に，植物病原菌の体外毒素が培地内から抽出される場合，非常に培養日数の経過した沪液から得られるのが普通で，病原菌の寄主侵入時間あるいは蔓延時間と培地内での毒素生成との時間的ずれが問題になつているが，蓚酸はこの点培養初期にも分泌が認められた．

(2) 蓚酸ならびに培養沪液によるペクチン分解　本菌が蓚酸を分泌する以上，それが中葉成分の分解に役立つか否かを知る必要がある．第1図は蓚酸の濃度と pectin 分解との関係を示したものであるが，この分解は0.1%前後の蓚酸で著しく，それ以上の濃度ではあまり分解が進まない．このことは，菌の分泌する蓚酸量がごく少ないとしても，かなりの pectin 分解を起すと考えてよい．また第1表に示す如く，培養沪液を熱処理して pectic enzyme を失活せしめても pectin 分解がみられるから，これにはその内に存在する蓚酸の作用が多分に関係しているのではないかと思われる．前報[2]における pectic enzyme 活性の測定は，基質が分解されて生じた還元力で表わしたが，その場



第1図　蓚酸の濃度とペクチン分解度との関係

第1表　培養に伴う depolymerase 活性の変動

培養日数	5		10		15		20	
区別	A*	B**	A	B	A	B	A	B
培養沪液	1′24″	1′34″	1′36″	1′22″	1′32″	1′26″	1′20″	1′38″
加熱沪液	2′36″	22″	2′32″	26″	2′32″	26″	2′30″	28″
対照	2′58″							

* 降下時間　** 対照との降下時間の差

合の pectic enzyme とは，pectin の粘度降下よりも還元力の増大が著しく，殆んど完全に galacturonic acid に分解する polygalacturonase を表わしたものと思われ，培養5日後ではその作用が全くみられない[2]．しかし pectin の粘度降下は培養5日後の酵素液において既に著しいから，この場合 pectin は oligogalacturonide となり，galacturonic acid までは分解しない depolymerase 作用によると考えてよい[7]．すなわち本菌の分泌する pectic enzyme は，培養初期には depolymerase の分泌がみられ，10日後になると depolymerase と共に polygalacturonase の分泌があると考えられる．加熱培養沪液の pectin 分解力は培養沪液に比べて極めて弱く，pectin 分解は多くは pectic enzyme によるけれども，蓚酸も一部は分解に関係していると思われる．

以上，本研究シリーズにおいて，現在までに得られた知見をとりまとめて病原菌側からみた罹病性を解析すると次のように要約される．

1. 本菌はその発育に biotin, thiamine, Zn, Fe, Mn などの微量を必要とするが[1]，解剖学的所見にみられるようにただ細胞間隙を伸長するだけではこれらは摂取出来ないと思われ，細胞内容物の吸収利用があると考えられる．

2. 細胞内容物の吸収を可能にする細胞膜の分解酵素として，菌は cellulase, xylanase を分泌するが，この分泌は菌を培養した場合発育初期に著しいから，寄主侵入及び蔓延時間とのずれがない[2]．

3. しかし本病々斑には崩壊部がないから，細胞膜全体の分解は行われず，その一部分が分解されているに過ぎないと思われる．しかし細胞膜にはミセル空隙があるから病原菌は必ずしも細胞膜の分解がなくてもミセル空隙を通つて滲透的に細胞内容物を吸収しうるかも知れない．しかし，寄主細胞は予め殺されているから，この透過性はなくなつていると予想される．

4. 寄主侵入直後の少数の寄主細胞の殺生には，発芽管の延長である菌糸自体の持つている energy で行われる．すなわちその時期までの pectic enzyme, cellulase, xylanase あるいは蓚酸などの分泌は，外界（寄主）からの養分に依存せず，分生胞子自体の持つていた内部 energy によつていると思われる．

5. このような侵入菌糸の通つた細胞は，その物理的傷害のみによつても死に至り，さらにその周囲の細胞も相互的動的平衡の破壊によつて変性を来す．ガラス粉末や微針で1細胞を機械的に傷つけても，その周りの細胞も変性褐変を引きおこす事実[15]はこの推定を裏ずける．

6. このようにして，殺された細胞は菌の栄養源となるが，ここで吸収された養分はまた，蓚酸や酵素生成の前駆物質となる．秋落稲は根腐れのために，窒素やその他の吸収が悪く，葉の蛋白合成はさまたげられているので，多くは可溶性窒素となつている[3]．このように最初から菌の利用可能な可溶態の養分が多いと，菌はそれだけ多量に吸収することができ，上述の酵素などの分泌が盛んとなり，大型病斑出現の一要因となつている[4]．

7. 拡大を停止した病斑内の菌糸は皆生存しているから[3]，菌が死ぬためにあるいは殺されるために病斑拡大が

止るのではない．従つて前述の酵素などの分泌がなくなるか，不活性化するかあるいはまた分泌しても分解を受けるべき寄主の基質が変質しているというような点が病斑拡大停止の最終過程ではないかと思われる[5]．しかし病原菌側のみから見た罹病性の解析は，このような考察にはまつたく無力であり，ここに寄主と病原菌との接触場面の時間的な，動的観察が必要になつてくる．

3. 摘　　要

稲胡麻葉枯病菌は菌体外に蓚酸を分泌するが，蓚酸はペクチン分解作用を示す．従つて稲葉の中葉成分の分解には菌の分泌する pectic enzyme と共に蓚酸がこれに関与していると考えられる．また菌の培養初期における pectic enzyme は depolymerase であつて，polygalacturonase は培養がやや進んでから分泌されるものと思われる．

引　用　文　献

1. 浅田泰次：日植病報，21：68，1956.
2. ————：同上，21：191，1956.
3. ————：同上，22：103，1957.
4. ————：植物病害研究，63(3)：109～113，1959.
5. ————：日植病報，22(4-5)：178～182，1957.
6. Foster, J. W.: Chemical activities of fungi. p. 326, 1949.
7. 福本寿一郎：赤堀編，酵素研究法，2，p.163，1956.
8. 逸見武雄：植物治病学汎論，p. 326，1926.
9. 樋浦　誠・永田幸雄：日植病報，13：68，1949.
10. ————・黒田佐俊：同上，14：44，1950.
11. 黒田佐俊・樋浦　誠：同上，14：94，1950.
12. ————：農業技術，11：255，1956.
13. 飯田　格・古山　清・綾　正弘：日植病報，15：178，1951.
14. 永田幸雄・林　金雄：農化，30：248，1956.
15. 高橋喜夫：栃内，福士両教授還歴記念論文集，p. 245，1955.

Summary

From the writer's observations the oxalic acid secretion by hyphae was detected in *Cochliobolus miyabeanus*, the causal fungus of the *Helminthosporium* blight of rice plants. It seems that pectin is decomposed by oxalic acid. Consequently the decomposition of middle lamellae of parenchymatous cells may be caused by the action of both oxalic acid and pectic enzymes secreted by this fungus. At the earlier stage of the artificial culture of this fungus, however, depolymerase may be secreted and the polygalacturonase secretion occurs in the later stage.

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